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Sreelekshmy S.G
Dept of Zoology,
Fatima Mata National College,
Kollam – 691001, Kerala.

Miranda M.T.P
Dept of Zoology,
Fatima Mata National College,
Kollam – 691001, Kerala.

D'cruz F.G
Dept of Zoology,
Fatima Mata National College,
Kollam – 691001, Kerala.

Rajesh B.R
Dept of Biotechnology,
The Cashew Export Promotion Council of India Laboratory & Technical Division, Kollam – 691001, Kerala.

Correspondence:
Sreelekshmy S.G
Dept of Zoology,
Fatima Mata National College,
Kollam – 691001, Kerala.

Utility of biomarkers in the marine catfish A*rius nenga* – A common food fish of Kerala – for Environmental Assessment

Sreelekshmy S.G, Miranda M.T.P, D'cruz F.G, Rajesh B.R

Abstract
In the present work, the utility of biomarkers in fish for assessing the environmental status was evaluated. The Chavara – Panmana coastal belt in Kollam, Kerala receives effluents from the major industries located along the coast. Histological investigation of various tissues (gill, liver and kidney) of the common food fish *Arius nenga* – a resident species – reveal severe alterations such as fusion of gill lamellae, hypertrophy and hyperplasia of epithelial cells and severe degeneration, aggregation of melano macrophage cells (MMC) in the liver and kidney. Significant haematological changes such as decrease in Hb, PCV, RBC and MCHC levels and increased WBC, MCH and MCV levels were observed.

Keywords: Biomarkers, histological alterations, hematological response, *Arius nenga,* heavy metals.

1. Introduction
Two common strategies to assess exposure to environmental toxicants are to measure chemical concentrations in the environment and examine the presence of certain species that are known to be sensitive to pollution. The two strategies can sometimes give conflicting results. It is possible that there are elevated levels of contaminants but no biological effect due to low bio availability. Furthermore, chemical measurements only give information about those chemicals that are included in the analysis and abundance of species can vary due to many factors that are not linked to pollution. An alternative or complementing strategy for environmental assessment is to examine sub – organism responses (biomarkers) which includes physiological and biochemical variables. Biomarkers indicate exposure to contaminants and health impairment in individuals. Because the initial response to pollution is assumed to occur at low levels of biological organization, biomarkers are expected to act as early warning signals. Furthermore, biomarkers may provide a mechanistic link between exposure and effects. There is, however little evidence that link biomarker responses to effects at higher levels. In addition, confounding factors such as migration and age, can complicate the interpretation of results.
In the present work, the utility of biomarkers in fish for assessing the environmental status was evaluated. Fish are suitable for assessing the environment as they can be found in most aquatic environments and play a major ecological role in aquatic food webs.
The Kerala Minerals and Metals Ltd (KMML) is a major industry located in Chavara, Kollam, Kerala. Highly acidic effluents from the factory containing significantly high concentrations of Titanium, Lead, Chromium and Cadmium are discharged into the Arabian sea at two points viz behind the Mineral Separation plant (MS plant) at Anchumanakal and at Uppukunnu, 2km from the main plant [1]. The marine catfish *Arius nenga* a common edible food fish extensively found along the Kerala coast in evaluating the utility of biomarkers for environmental assessment.

2. Materials and Methods
2.1 Study Area
Chavara Panmana region (9^0 5' N & 76^0 31'E) of Kollam, Kerala which receives the effluent discharge from the KMML titanium dioxide factory.

2.2 Sampling stations and sampling protocol.
Three sampling stations (S1, S2 & S3) were selected at a distance of 10m, 100m and 1000m from the shore line along one transect from the point of effluent entry into the sea. Sampling was done in March 2014.
Water samples (surface and bottom) were collected from the three sites in March 2014. Bottom water samples were collected using a Nessler's bottom water sampler. Experimental fishes were randomly collected from the impacted site while control fishes were obtained from the presumably pristine coast of Arthunkal, Kerala.

2.3 Hydrological Parameters
Physico-chemical analysis such as temperature, pH, salinity, Dissolved Oxygen, and heavy metal content (Fe, Pb, Cd, Cu, Ni, Zn, Cr, & V) of the water samples were carried out. Temperature of the seawater was recorded using a mercury thermometer. PH was recorded using a calibrated pH digital pen. Salinity of seawater samples was measured using a water analyser (Systronics model – 371). Dissolved Oxygen was estimated using Winkler's method, 1883. Heavy metals were estimated using ICP-OES [2].

2.4 Analysis of *A. nenga*
Heavy metal content in the tissue (gill, liver and kidney) of the experimental fish was carried out. 0.5 gm of sample was digested in a mixture of 5ml nitric acid and 2ml of perchloric acid and were quantified using ICP-OES [3].

2.5 Histological studies
Fishes were dissected and tissue samples of gill, liver and kidney were removed and fixed in 4 % formaldehyde. After fixation, tissue was dehydrated in ethanol, equilibrated in xylene, and embedded in paraffin according to standard histological techniques. Sections of 5-7 µm were cut and stained with haematoxylin and eosin and observed under a light microscope. Histological changes induced by the heavy metals were photographed using Olympus CX21i microscope.

2.6 Haematological studies
Blood was drawn from the posterior caudal peduncle and collected in heparinized bottles for red blood cell count (RBC), haematocrit (PCV), haemoglobin (Hb) and white blood cell count (WBC). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined [4].

3. Results and Discussion
3.1 Analysis of water samples
A sufficient load of DO in the surface and bottom water was recorded at the three stations. The difference in the DO, water temperature and pH at the sampling stations are given in **Table 1.**

Table 1: Hydrological parameters

	Surface water			Bottom water		
	S1	S2	S3	S1	S2	S3
Temp. (^{0}c)	28.4 ± .1	28.2 ± 0	28.0 ± 0	27.6 ± .2	27.0 ±.2	27.4 ± 0
pH	6.0 ± .05	6.2 ± .1	6.8 ± 0	6.2 ± .1	6.3 ±.1	6.7 ±.09
Salinity (ppt)	33.6 ± 0	33.5 ± 0	32.6 ±.1	33.4 ± .0	33.3 ± .1	32.8 ± 0
DO (mg/l)	4.2 ± 0	4.8 ± .1	4.7 ± .1	4.2 ± .1	4.6 ±.1	4.7 ± 0

3.2 Distribution of dissolved heavy metals in surface and bottom water
A wide fluctuation was observed in the distribution of heavy metals. Bottom water samples contained comparatively higher loads of heavy metals **(Table 2)**. Heavy metal accumulation in surface water at S1, S2 and S3 were in the following descending order Fe > V > Zn > Ni > Pb >Cu > Cr > Cd. Mercury and arsenic were not detected. In the bottom water, Fe recorded the highest concentration while Cd and Cu recorded lowest concentration.

Table 2: Heavy metal concentration in Surface and Bottom water (µg/l)

	Surface water			Bottom water		
	S1	S2	S3	S1	S2	S3
Fe	2402.17	1801.30	1752.47	2410	1787.53	1871.20
V	128.20	105.77	89.43	128.5	127.60	87.33
Z	8.50	14.50	16.60	9.7	17.77	12.67
Ni	2.17	2.60	4.30	2	2.27	6.40
Pb	15.43	12.43	17.37	17.23	16.03	20.93
Cd	0.39	0.25	0.29	0.67	0.13	0.32
Cu	0.00	2.20	2.33	0.00	2.73	3.17
Cr	0.00	0.00	0.66	0.00	0.53	1.70

3.3 Bioaccumulation of Heavy metals in tissues of *A. nenga*.
The concentration of Fe, Cu and Mn was high in all the tissues under study **(Figure 1)**.

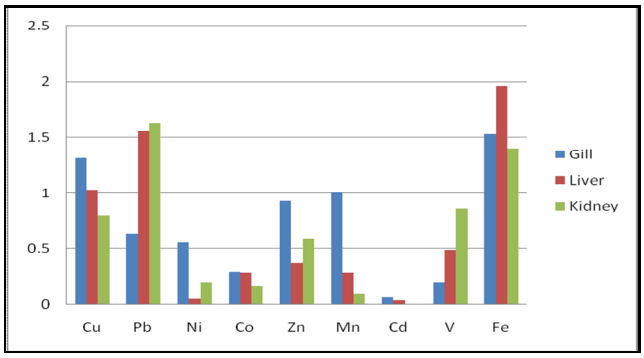

Fig 1: Graph showing the concentration of heavy metal in A.nenga (µg/g)

3.4 Histopathological Alterations
3.4.1 Kidney: Kidney showed edema in the epithelial lining of renal tubules and degeneration and vacuolation of renal cells. Moreover, many necrotic areas and many pyknotic nuclei as well as swollen proximal epithelial cells with necrotic nuclei were noticed **(Figure 2).**

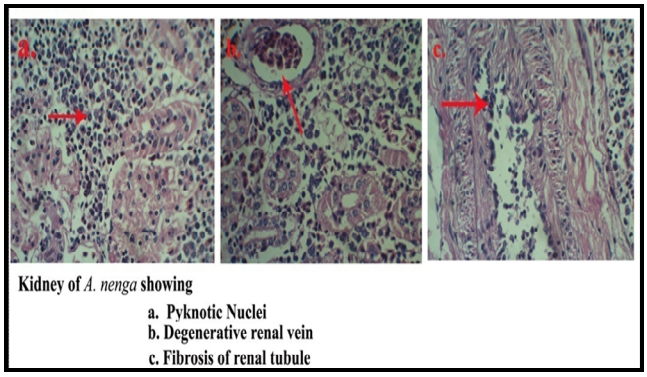

Fig 2: Histological alterations in the kidney tissue of *A.nenga*

3.4.2 Liver: Degeneration of hepatocytes, hemorrhage, vacuolization and hypertrophy were observed **(Figure 3).** The hepatocytes appeared swollen which resulted in its rupturing. Increased accumulation of pyknotic nuclei was observed in majority of hepatic cells and the metal – binding proteins were accumulated in the nuclei.

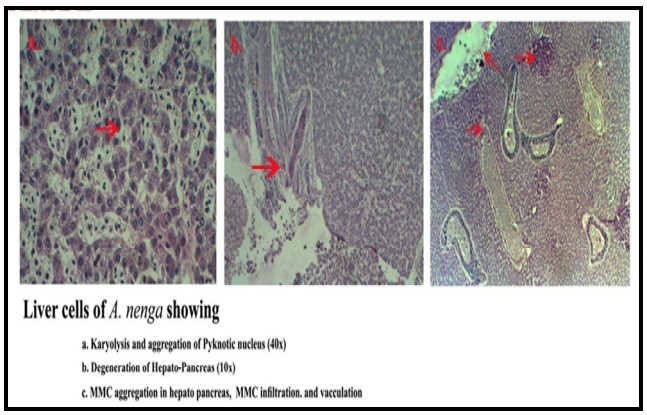

Fig 3: Histological alterations in the liver tissue of *A.nenga*

3.4.3 Gill: The histological analysis of gill tissue showed lifting of the lamellar epithelial cells from the basement membrane due to penetration of fluid. Congestion and severe hemorrhage was prominent in the primary lamellae. The lesions observed in the gills included curling and fusion in secondary lamellae, lamellar swelling, edema and blubbing in secondary and primary lamellae and in the gill filaments. Lamellar fusion, hyperplasia, hypertrophy of epithelial cells, necrosis of different lamellar and filament cells were also observed. Moreover, hyperplasia of the epithelial cells decreases the interlamellar space **(Figure 4).**

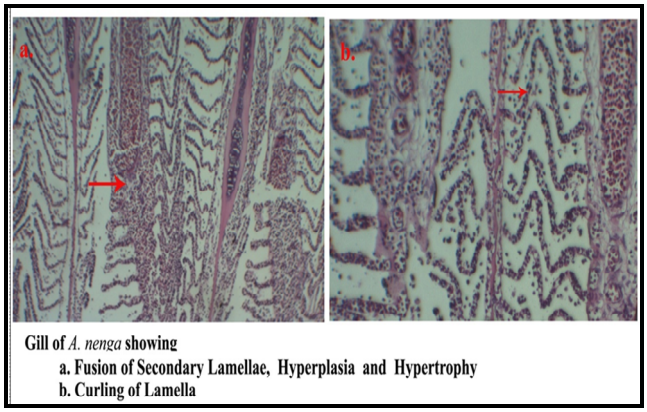

Fig 4: Histological alterations in the gill of *A.nenga*

3.5 Haematological Indices

Haematological parameters are considered pathophysiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants.

Quantitative changes in the blood parameters were observed **(Figure 5)**. Significant decrease in the Hb, PCV, RBC and MCHC levels were observed ($p<0.01$). A decrease in these values can be attributed to the destruction of RBC caused by the toxic effect of heavy metals whereas the WBC, MCH and MCV levels increased in concentration. This increase in the WBC count is due to leucocytosis which causes to leukemia.

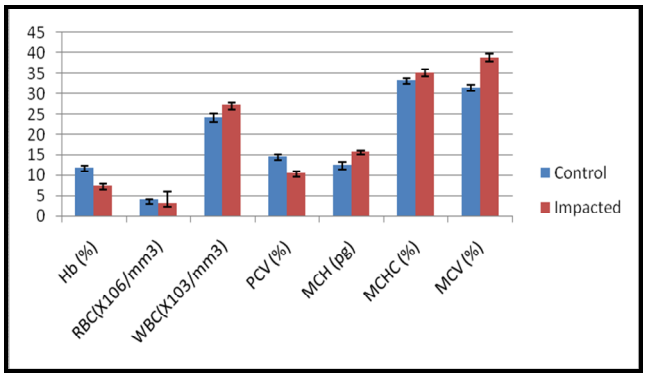

Fig 5: Graph showing the Haematological variations in *A. nenga*

The use of toxicological and biochemical responses to detect the effects of environmental contamination is of extreme importance in the present scenario. The biomarkers used in the present study for the first line evaluation of environmental pollution were found to be very effective.

Histopathological alterations in *A. nenga*, can be used as a sensitive model to monitor aquatic pollution. The heavy metal load in the effluent from the KMML titanium dioxide industrial factory poses a serious threat to the resident fauna as evidenced by the present study. Severe histopathological alterations were observed in the *Lates calcarifer* exposed to sublethal concentration of individuals and mixed metals [5]. They have recommended bioindicators for the first line evaluation of environmental pollution. Significant hematological alterations and behavioral changes observed could potentially disrupt the survivability of the fish in their natural environment. Changes in the blood cell number, alterations in blood cell dimension, reduction in Hb, variations in PCV, MCV, MCHC and MCH values denote the destruction caused to blood cells, diminished production of hematocytes and increased defensive usage of blood cells which indicate the extent of the toxicity stress caused to *A. nenga*.

4. Conclusion

Biomarkers in fish can be a useful tool to evaluate environmental pollution in many, although not all, situations. The use of farmed fish can improve the precision in the measurements, but some ecological relevance will be lost and certain confounding factors may be introduced. The choice between farmed or feral fish depends on the situation and what questions needs to be answered. The results from biomarkers analysis are most useful when data from other methodologies can be included in the interpretation. Future work should focus on how different methods can be integrated to get an effective and more reliable assessment of environmental conditions.

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6. References

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